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Research Note

Egg load and body size of lab-cultured *Cotesia marginiventris**

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Abstract. The egg load of lab-cultured *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), a solitary koinobiont endoparasitoid of noctuid caterpillars, was determined in this study. Information on egg load may provide clues to more efficient *in vivo* rearing of *C. marginiventris*. I tested the hypothesis that egg load, defined as the number of mature oöcytes (i.e., fully chorionated eggs) found in adult females, was related to body size. *Cotesia marginiventris* females possessed two ovaries and two ovarioles per ovary; mature eggs were found in ovaries and oviducts. Newly-emerged females held an average of 149 mature eggs. Immature eggs were slightly visible in the distal portions of the ovarioles; they were not counted. Egg load was marginally related to body size (i.e., hind tibia length). The results of this study suggest that (1) body size can sometimes predict egg load or potential fecundity of lab-cultured *C. marginiventris* and (2) an efficient rearing system that exploits the potential fecundity of *C. marginiventris* might involve using young females and allowing them to oviposit in new hosts, each day, for up to a week.

Key words: *Cotesia*, *Spodoptera*, egg load, endoparasitoid, oviposition strategy, pro-synovigenic, rearing

Introduction

Cotesia marginiventris (Cresson) (Hymenoptera: Braconidae) is native to the West Indies (Muesbeck, 1921) and is currently distributed in North, Central, and South America. It is an important solitary, koinobiont endoparasitoid of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), and other pestiferous noctuids (McCutcheon, 1987; Ruberson et al., 1994; Novoa and Luna, 1996).

There has been some interest in using *C. marginiventris* for augmentative biological control of lepidopterans on vegetables grown in greenhouses in Canada and Spain (Gillespie et al., 1997; Urbaneja et al., 2002). A prerequisite to augmentative (inundative) releases is the production of large quantities of quality insects (Nordlund, 1998). Refinement and improvement of *in vivo* methods of rearing *C. marginiventris* are needed, especially since *in vitro* methods have not been fully developed.

Knowledge of the oviposition behavior of *C. marginiventris* may provide insights on designing efficient systems for *in vivo* rearing of this parasitoid. In order to more-fully understand the oviposition behavior of *C. marginiventris*, I examined the egg load of newly-emerged females in this study. Egg load can be defined as the number of mature oöcytes (i.e., fully-chorionated eggs) found within the ovaries and oviducts of an insect at any given moment in its lifetime; it has been used as a measure of fitness in a number of insects (Heimpel and Rosenheim, 1998; Jervis and Ferns, 2004). Emergence with a high percentage of mature rather than immature eggs would seem advantageous to short-lived species that generally expend much of their egg load early-on in adult life; whereas, emergence with a low percentage of mature eggs would seem advantageous to species that oviposit a limited number of eggs over a longer period of time (Ellers and Jervis, 2003). Species that emerge with a full complement of mature eggs are strictly proovigenic (e.g., *Cotesia flavipes* (Cameron)) and those that emerge with few or no mature eggs are strictly synovigenic (e.g., *Microtonus hyperodae* Loan) (Jervis et al., 2001, 2003). There is likely a continuum of ovigenic strategies in the parasitic Hymenoptera, ranging between the extremes of strict proovigeny and synovigeny (Quicke, 1997). Species that fall somewhere between the extremes have been called pro-synovigenic; they emerge with a considerable number of mature eggs and are capable of maturing more eggs as the initial supply is depleted (Quicke, 1997).

It is unknown whether the initial egg load of *C. marginiventris* females is related to body size, but egg load or potential fecundity has been related positively to body size of many pterygote insects (Wickman and Karlsson, 1989; Hoňek, 1993; Mills and Kuhlmann, 2000; Ellers and Jervis, 2003; Jervis et al., 2003). This relationship may not always hold true for very small-sized, short-lived parasitoids (Ellers and Jervis, 2004), lab-cultured parasitoids (Riddick, 2005) or lab-cultured predators (Mohaghegh et al., 1999).

In this study, the egg load of newly-emerged *C. marginiventris* females was determined for the first time and the hypothesis that egg load was related to body size was tested. This research should help define the potential fecundity of *C. marginiventris* and provide clues necessary for efficient propagation of this parasitoid from noctuid hosts.

Materials and methods

Insect cultures

Cotesia marginiventris was reared at the USDA-ARS, Biological Control and Mass Rearing Research Unit (BCMRRU), Mississippi State, MS for more than 150 continuous generations. The original parasitoids came from a colony maintained at an USDA-ARS facility in Tifton, GA. The culture at BCMRRU was maintained by exposing late first to second instar *S. exigua* larvae, reared on a meridic diet (after King and Hartley, 1985), to *C. marginiventris* females for 24 h in a 'sting' box (45.7 cm × 66.0 cm × 8.9 cm, W × L × H; 18.9 l clear plastic) provisioned with pure honey and sterile water, in cotton pads. Parasitized larvae were placed within polystyrene rearing trays (with diet) and held in an environmental room (27 °C, 60–70% RH, and 16 h photophase). After 2 wk, trays were removed and *C. marginiventris* cocoons (i.e., pupae) were harvested.

Egg load vs. body size relationship

An experiment was designed to test the hypothesis that egg load was related to body size of lab-cultured *C. marginiventris*. This study consisted of one trial (7 October, 2004); representing the date that a batch of *C. marginiventris* cocoons were harvested from rearing trays. The sample included 199 cocoons, which were placed, individually, inside glass shell vials (≈ 13 mm × 100 mm), stoppered with cotton. No food or water was provided in the vials. Samples were held in an environmental room (at 27 °C, 60–70% RH, and 16 h photophase). Vials were checked daily for emergence of adults; 0-d-old females were collected and placed within a counter-top freezer (−18 to −20 °C), until dissection. Emergence was completed in six days; a total of 155 adults (76 females, 79 males) emerged successfully from cocoons.

For dissection, each female was placed on a glass microscope slide in several drops of saline solution (i. e., 6.5 gm NaCl per liter) and observed under a stereo-zoom dissecting microscope (60--90×).

Using two # 0 insect micropins, the abdomen of each female was teased apart and the reproductive system was exposed. A glass coverslip was placed over the specimen and the mature eggs in the ovaries and lateral oviducts were counted. Immature eggs were not counted; they were barely discernable in the distal portions of the ovarioles. The body of the female (minus the reproductive system) was placed on another glass slide, in saline solution, and covered with a coverslip. The hind tibia was used to estimate body size. This character has been used previously for estimating body size of other parasitoids (Olson and Andow, 1998; Mills and Kuhlmann, 2000; Riddick, 2005). A digital imaging software program, Image-Pro Plus (1999), was used to make length measurements (in mm), with the aid of the dissecting microscope and camera system. The number of mature eggs found in each 0-d-old female was determined and then related to the length of the hind tibia. A total of 76 females were dissected and measured in this experiment. Individual females were considered as sampling units.

Simple linear regression (using the least squares method) was used to determine if there was a significant relationship between egg load and body size. Transformation of data, prior to analysis, was not necessary because the requirements of normality and homogeneity of variances were met (see Zar, 1999). The regression analysis indicated a significant relationship when $p < 0.05$. SigmaStat (2004) software was used for the analysis.

Results and discussion

Egg load and body size

Each *Cotesia marginiventris* female possessed two ovaries with two ovarioles per ovary. Mature eggs were found in the ovaries and lateral oviducts. The total mean ± SEM number of mature eggs (i.e., egg load) in each newly-emerged (0-d-old) virgin female was 149.0 ± 1.8 eggs (range, 114–176 eggs; n, 75 females). Usually the same number of mature eggs was found in both ovaries/lateral oviducts. Egg load was marginally related to body size; there was a significant relationship between egg load and female hind tibia length [y *(egg load)* = 5.7 + 162.9x (*hind tibia length*); $r^2 = 0.07$; $F = 5.5$; df = 1, 73; p = 0.02; Figure 1]. The mean ± SEM hind tibia length was 0.88 ± 0.003 mm (range, 0.81–0.94 mm; n, 75 females). Note that r^2 was very low, which suggests that the straight line does not account

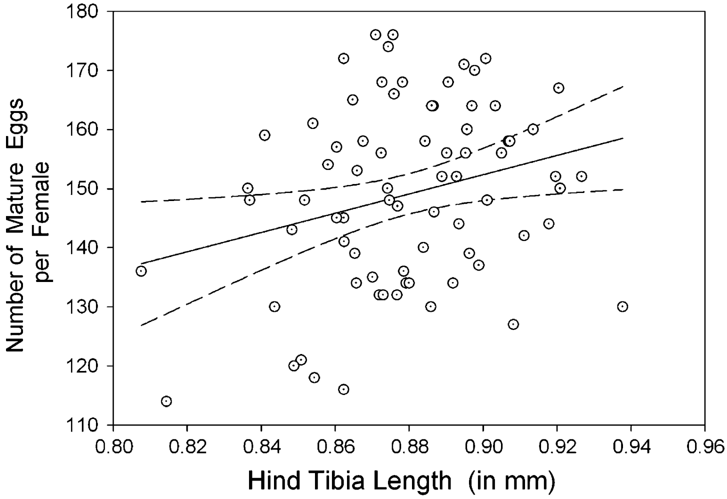

Figure 1. Relationship between *C. marginiventris* egg load vs. body size (length of hind tibia, in mm). n, 75 newly-emerged, virgin females. The regression line defines the relationship between egg load (y) vs. tibia length (x). Simple linear regression; $y = y_o + ax$; y_o, 5.7; a, 162.9; $r^2 = 0.07$; n, 75 observations. The solid line and dashed lines represent the regression line and 95% confidence intervals, respectively.

for much of the variability in egg load. Thus, the straight line relationship between egg load and body size is weak.

The fact that *C. marginiventris* egg load ranged from 114 to 176 mature eggs suggests that considerable variation in egg production can occur between females reared under identical conditions. This variation only slightly reflects differences in body size in this study. Perhaps, some variation in egg load was a reflection of the quality and body mass of the host. The impact of host (*S. exigua*) nutrients on parasitoid development or egg load of female progeny has not been determined. Interestingly, a weak functional relationship between egg load and body size was apparent, despite the continuous rearing of *C. marginiventris* for many generations in the laboratory.

Mohaghegh et al. (1999) found no relationship between fecundity and body length of females of a Brazilian strain of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae), which had been lab-cultured for at least five generations. Riddick (2005) found that egg load was not related to body size of *Anaphes iole* Girault (Hymenoptera: Mymaridae), which had been lab-cultured for more than six consecutive years. Contrary to these studies, body size can be used to predict the egg load or potential fecundity of lab-cultured *C. marginiventris* females.

The presence of an average of 149 mature eggs in the ovaries and lateral oviducts of newly-emerged *C. marginiventris* females highlights the storage capacity of this species. Flanders (1942) indicated that females of some *Apanteles* Foerster species are capable of storing many mature eggs, prior to oviposition, in the paired oviducts and in the base of the ovary, which may be correlated with the ability to oviposit a large number of eggs into hosts within a short period of time. A novel rearing method that gave *C. marginiventris* access to hosts for just 24 h in unicellular, polystyrene trays yielded an average of 20 pupae per female (Riddick, 2004). Tillman (2001) indicated that the average realized fecundity of lab-cultured females exposed to an abundance of early instar *S. exigua* larvae over a lifetime (up to 10 days in 'sting cages') was 173 pupae. Most progeny of normal sex ratios (46–50% females) were produced during the second through fifth days of exposure to hosts (Tillman, 2001). Hence, several days rather than one day of exposure to new (i.e., un-parasitized) hosts would exploit the potential fecundity of *C. marginiventris* and yield suitable numbers of female offspring.

The fact that some developing eggs were present in the distal portions of the ovarioles suggests that further egg maturation can occur post-emergence. Maternal age and food (i. e., honey, for maintenance) might stimulate further egg maturation, thereby increasing the egg load of *C. marginiventris* before oviposition begins (EWR, unpublished data). In contrast, Quicke (1997) believes that newly-emerged pro-synovigenic parasitoids must begin ovipositing into suitable hosts before egg maturation can recommence. Lab-cultured *C. marginiventris* females are most likely pro-synovigenic, since they emerge with a great quantity of mature eggs, ready for oviposition, and also some immature eggs within the ovarioles.

In conclusion, this study suggests that (1) body size can sometimes predict egg load or potential fecundity of lab-cultured *C. marginiventris*, and (2) an efficient *in-vivo* system that exploits the potential fecundity of *C. marginiventris* might involve using young females and allowing them to oviposit in new hosts, each day, for up to a week.

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References

Ellers, J. and M.A. Jervis, 2003. Body size and the timing of egg production in parasitoid wasps. *Oikos* 102: 164–172.

Ellers, J. and M.A. Jervis, 2004. Why are so few parasitoid wasp species pro-ovigenic? *Evol. Ecol. Res.* 6: 993–1002.

Flanders, S.E., 1942. Oösorption and ovulation in relation to oviposition in the parasitic Hymenoptera. *Ann. Entomol. Soc. Am.* 35: 251–266.

Gillespie, D., G. Opit, R. McGregor, M. Johnston, D. Quiring and M. Foisy, 1997. Recommendations for the use of the parasitic wasp, *Cotesia marginiventris*, (Hymenoptera: Braconidae) for biological control of cabbage looper, *Trichoplusia ni*. Pacific Agri-Food Res. Centre (Agassiz), Tech. Rep. 142: 1–2.

Heimpel, G.E. and J.A. Rosenheim, 1998. Egg limitation of parasitoids: a review of the evidence and a case study. *Biol. Control* 11: 160–168.

Hoňek, A., 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* 66: 483–492.

Image-Pro Plus, 1999. *Reference Guide for Windows, Version 6.0*. Media Cybernetics, Silver Spring, MD.

Jervis, M.A. and P.N. Ferns, 2004. The timing of egg maturation in insects: ovigeny index and initial egg load as measures of fitness and of resource allocation. *Oikos* 107: 449–460.

Jervis, M.A., G.E. Heimpel, P.N. Ferns, J.A. Harvey and N.A.C. Kidd, 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *J. Anim. Ecol* 70: 442–458.

Jervis, M.A., P.N. Ferns and G.E. Heimpel, 2003. Body size and the timing of egg production in parasitoid wasps: a comparative analysis. *Funct. Ecol.* 17: 375–383.

King, E.G. and G.G. Hartley, 1985. Heliothis virescens. In: P. Singh and R.F. Moore (eds), *The Handbook of Insect Rearing*. II Elsevier, NY. pp. 323–328.

McCutcheon, G.S., 1987. Potential of the parasitoid *Cotesia marginiventris* as a biocontrol agent of lepidopterous larvae in soybean. Ph. D. dissertation, Univ. Georgia, Athens.

Mills, N.J. and U. Kuhlmann, 2000. The relationship between egg load and fecundity among *Trichogramma* parasitoids. *Ecol. Entomol.* 25: 315–324.

Mohaghegh, J., P. De Clercq and L. Tirry, 1999. Effects of rearing history and geographical origin on reproduction and body size of the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae). *Eur. J. Entomol.* 96: 69–72.

Muesbeck, C.F.W., 1921. A revision of the North American species of Ichneumon-flies belonging to the genus *Apanteles*. *U. S. Nat. Mus. Proc.* 58: 483–576.

Nordlund, D.A., 1998. Capacity and quality: keys to success in the mass rearing of biological control agents. *Nat. Enemies Ins.* 20: 169–179.

Novoa, M.C. and M.G. Luna, 1996. Parasitism, survivorship, sex ratio, and developmental time of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), parasitizing *Rachiplusia nu* (Guenée) (Lepidoptera: Noctuidae) larvae in soybean crops in Argentina. *Acta Entomol. Chilena* 20: 23–28.

Olson, D.M. and D.A. Andow, 1998. Larval crowding and adult nutrition effects on longevity and fecundity of female *Trichogramma nubilale* Ertle and Davis (Hymenoptera: Trichogrammatidae). *Environ. Entomol.* 27: 508–514.

Quicke, D.L.J., 1997. *Parasitic Wasps*. Chapman and Hall, London.

Riddick, E.W., 2004. Production of the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae) in unicellular rearing trays using the host *Spodoptera exigua* (Lepidoptera: Noctuidae). *J. Entomol. Sci.* 39: 362–372.

Riddick, E.W., 2005. Egg load of lab-cultured *Anaphes iole* and effects of mate presence and exposure time on load depletion. *BioControl* 50: 53–67.

Ruberson, J.R., G.A. Herzog, W.R. Lambert and W.J. Lewis, 1994. Management of the beet armyworm (Lepidoptera: Noctuidae) in cotton: role of natural enemies. *Fla. Entomol.* 77: 440–453.

SigmaStat, 2004. *Getting Started Guide, Version 3.0*. SYSTAT Software Inc., Richmond, CA.

Tillman, P.G., 2001. Factors affecting parasitization of *Spodoptera exigua* (Lepidoptera: Noctuidae) and sex ratio of the parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae). *J. Entomol. Sci.* 36: 188–198.

Urbaneja, A., J. van der Blom, L. Lara, R. Timmer and R. Blockmans, 2002. Utilización de *Cotesia marginiventris* (Cresson) (Hym.: Braconidae) para el control biológico de orugas (Lep.: Noctuidae) en el manejo integrado de plagas en pimiento bajo invernadero. *Bol. San. Veg. Plagas* 28: 239–250.

Wickman, P.-O. and B. Karlsson, 1989. Abdomen size, body size and the reproductive effort of insects. *Oikos* 56: 209–214.

Zar, J.H., 1999. *Biostatistical Analysis*. 4th edn. Prentice Hall, NJ.